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EVALUATION OF WASTE CITRUS ACTIVATED SLUDGE IN POULTRY FEEDS

by

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16. ABSTRACT

Experiments were conducted on chick broilers and hens to determine the metabolizable energy of citrus sludge. A determination of metabolizable energy values showed that the values decreased as the level of citrus sludge in the diet increased. A series of protein levels were fed to day-old chicks for a 3-week period in an attempt to assay the protein quality of citrus sludge. As the level of sludge in the diet increased, its utilization decreased with an almost toxic effect being observed at higher concentrations.

Vitamin D_3 and citrus sludge were fed to day-old chicks to determine if the minerals contained in the sludge could be causing the destruction of Vitamin D_3 in the feed. Experiments were also conducted to evaluate the biological availability of phosphorus contained in the sludge. Citrus sludge was also examined as a source of pigmentation in egg yolks and in broilers.

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FOREWORD

When energy and material resources are extracted, processed, converted, and used, the related pollutional impacts on our environment and even on our health often require that new and increasingly more efficient pollution control methods be used. The Industrial Environmental Research Laboratory-Cincinnati (IERL-Ci) assists in developing and demonstrating new and improved methodologies that will meet these needs both efficiently and economically.

This report presents the results of a two and one half year study in which the liquid sludge from the waste treatment plant of a large citrus processing facility was evaluated as a supplement in poultry feeds. The overall purpose of the project was to develop a beneficial use of a food processing residual which is currently considered a waste material. The results of this study should be of interest to citrus processors, regulatory agencies and food researchers. For further information on this study, contact the Food and Wood Products Branch, Industrial Pollution Control Division, IERL-Ci.

David G. Stephan
Director
Industrial Environmental Research Laboratory
Cincinnati

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ABSTRACT

Liquid sludge from the waste treatment plant of a large citrus processing facility (Citrus World, Inc. of Lake Wales, Florida) was centrifuged and then hot-air dried for use in subsequent poultry feeding experiments.

An experiment was conducted with Cobb, color-sexed, broiler chicks to determine the metabolizable energy of citrus sludge. A modified basal diet containing 40 percent sucrose was employed according to the procedures of Hill *et al* (1960) with chromic oxide as an indicator.

A determination of metabolizable energy (ME) values showed that the values decreased as the level of citrus sludge in the diet increased. From this it was concluded that a meaningful energy value for the product could not be arrived at because of the depressed performance that resulted from increasing levels of sludge inclusion.

In two experiments, a graded series of protein levels were fed to day-old Cobb, color-sexed broilers for a three-week feeding period in an attempt to assay the protein quality of citrus sludge. At the lowest level of sludge addition, its protein availability appeared to be approximately 75 percent of that supplied by corn-soy. As the level of sludge in the diet increased, its utilization decreased with an almost toxic effect being observed at the highest level of addition.

A graded series of vitamin D_3 levels ranging from 200 to 1600 ICU and sludge levels of 0, 5, 10, and 15 percent were fed in two experiments to day-old, broiler chicks in order to determine if the iron or other heavy metals contained in the sludge could be causing vitamin D_3 destruction in the feed and, hence, depressed performance. It was found that addition of vitamin D_3 to the diet did not offset the depressing effect of sludge feeding upon body weights. Tibia ash data were not significantly influenced by any level of sludge or vitamin D_3. Thus it was concluded that the adverse effects of sludge addition were not related to vitamin D_3 destruction.

Duplicate experiments were conducted with day-old, broiler chicks to evaluate the biological availability of the phosphorus contained in citrus sludge. A modified corn-soy basal diet containing 0.34 percent phosphorus and 0.095 percent calcium was employed with a supplemental level of 0.05 percent phosphorus coming from sludge fed at three total calcium levels. Standard diets carried either 0.025 or 0.05 percent phosphorus from sodium acid phosphate, each with three total calcium levels. All sludge treatments produced body weights significantly below those of monosodium phosphate diets. The

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tibia ash values of two sludge treatments (0.52 and 0.58 percent calcium) did not differ significantly from any of the 0.05 percent phosphorus treatments from monosodium phosphate. The highest tibia ash value associated with sludge addition was 96 percent of that supported by the best monosodium phosphate treatment.

Citrus sludge was also examined as a potential source of pigmentation for broiler skin and egg yolks. In each of two trials, White Leghorn hens were fed a white corn basal, xanthophyll-free diet to deplete egg yolk pigmentation. Levels of 0, 2.5, 5.0, 7.5, or 10 percent citrus sludge were then provided along with yellow corn diets containing comparable amounts of xanthophyll. It was found that citrus sludge produced eggs with the same hue as yellow corn, although the xanthophylls in the product were apparently not as available as those of yellow corn. In market channels requiring further processing, this difference could be a problem; however, for table eggs it is as effective as yellow corn in pigmenting yolks.

Color-sexed broilers were used in two experiments to determine the effectiveness of citrus sludge as a pigmenting agent for broilers. Levels of 2.5, 5.0, 7.5, and 10 percent sludge were mixed into white corn diets, with a series of yellow corn diets also being fed to provide comparable levels of xanthophyll. Feeding citrus sludge resulted in broiler shanks and skins with the same hue and darkness as feeding yellow corn at equivalent xanthophyll levels. However, EP values indicated that the xanthophylls of yellow corn appeared to be more available than those in citrus sludge.

This report was submitted in fulfillment of Grant No. R-803997 by the Department of Poultry Science, University of Florida under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period July 13, 1976 to January 12, 1979 and work was completed as of December 29, 1978.

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CONTENTS

Foreword iii
Abstract iv
Tables vii
Acknowledgement ix

1. Introduction 1

2. Conclusions and Recommendations 4

3. Sludge Procurement and Drying 5

4. Metabolizable Energy of Citrus Sludge 7
   Experimental Procedure 7
   Results and Discussion 8

5. Citrus Sludge Protein Assay 10
   Experimental Procedure 10
   Results and Discussion 10

6. Citrus Sludge-Vitamin D_3 Interaction 13
   Experimental Procedure 13
   Results and Discussion 14

7. Phosphorus Assay of Citrus Sludge 18
   Experimental Procedure 18
   Results and Discussion 19

8. Evaluation of Citrus Sludge as an Effective Egg Yolk Pigmenting Ingredient 21
   Experimental Procedure 21
   Results and Discussion 23
      Experiment 1 23
      Experiment 2 26

9. Evaluation of Citrus Sludge as an Effective Broiler Skin Pigmenting Ingredient 27
   Experimental Procedure 27
   Results and Discussion 28
      Experiment 1 28
      Experiment 2 29

References 35

BEGIN LAST LINE OF TEXT
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3/8"

PAGE NUMBER
EPA-287 (Cin.)
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PAGE NUMBER
EPA-287 (Cin.) (4-76)

TABLES

Number		Page
1	Proximate Analyses of Citrus Sludge	2
2	Amino Acid profile of Citrus Sludge	2
3	Trace Mineral Analysis of Citrus Sludge	3
4	Pesticide Residue Analysis of Citrus Sludge	3
5	Check Analysis of Sludge Effluent	5
6	Metabolizable Energy of Citrus Sludge	7
7	Metabolizable Energy Per Kilogram of Citrus Sludge	9
8	Composition of Citrus Sludge Protein Assay Diets	11
9	Protein Assay Chick Performance	12
10	Diets Containing Graded Levels of Citrus Sludge To Which Varying Amounts of Vitamin D_3 Were Added	14
11	Citrus Sludge - Vitamin D_3 Interaction Chick Performance (3 weeks)	15
12	Performance of Chicks According to Vitamin D_3 Level Fed (3 weeks)	16
13	Daily Feed Intake of Chicks That Received Various Levels of Citrus Sludge and Vitamin D_3 (experiments 1 and 2)	16
14	Two-way Body Weight Results of Chicks That Received Various Levels Of Citrus Sludge and Vitamin D_3	17
15	Mortality of Chicks That Received Various Levels of Citrus Sludge and Vitamin D_3	17
16	Phosphorus Assay Basal Diet	18
17	Response of 3-week Old Chicks To The Feeding of Various Levels of Calcium and Phosphorus from Citrus Sludge and Standard Sources	19
18	Composition of Data For Experiments 1 and 2	22

BEGIN FIRST LINE OF TEXT HERE
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CENTER OF PAGE
TOP OF IMAGE AREA
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TABLES (CONTINUED)

Number		Page
19	Percent Production (Hen-day), Feed Efficiency, and Feed Consumption For Hens Fed Citrus Sludge or Yellow Corn at Equivalent Xanthophyll Levels in Experiment 1 and 2.	24
20	Visual Scores, DWL, EP, and LUM Values For Egg Yolks From Hens Fed Citrus Sludge or Yellow Corn Diets at Equivalent Xanthophyll Levels	25
21	Composition of Diets in Experiments 1 and 2 (Percent)	30
22	Body Weights and Feed Efficiencies For Broilers Fed Various Citrus Sludge And Yellow Corn Diets From 4-8 Weeks At Equivalent Xanthophyll Levels - Experiment 1.	31
23	DWL, EP, and LUM Values For Shank and Skin Samples From Broilers Fed Citrus Sludge or Yellow Corn Diets At Equivalent Xanthophyll Levels In Experiment 1	32
24	Body Weights and Feed Efficiencies For Broilers Fed Various Citrus Sludge and Yellow Corn Diets From 4-8 Weeks at Equivalent Xanthophyll Levels - Experiment 2.	33
25	DWL, EP, and LUM Values For Shank and Skin Samples From Broilers Fed Citrus Sludge or Yellow Corn Diets at Equivalent Xanthophyll Levels In Experiment 2.	34

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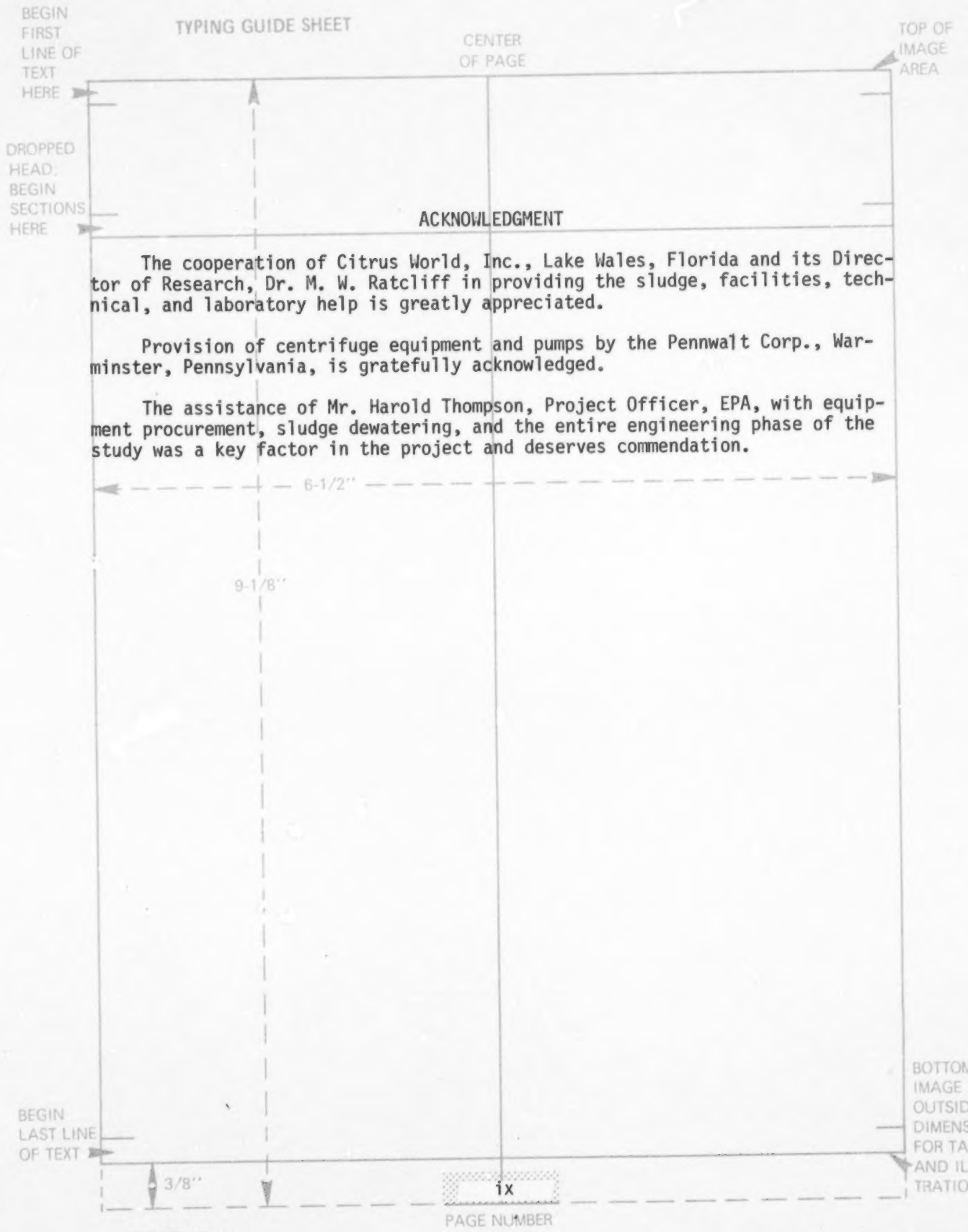
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The cooperation of Citrus World, Inc., Lake Wales, Florida and its Director of Research, Dr. M. W. Ratcliff in providing the sludge, facilities, technical, and laboratory help is greatly appreciated.
Provision of centrifuge equipment and pumps by the Pennwalt Corp., Warminster, Pennsylvania, is gratefully acknowledged.
The assistance of Mr. Harold Thompson, Project Officer, EPA, with equipment procurement, sludge dewatering, and the entire engineering phase of the study was a key factor in the project and deserves commendation.
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SECTION 1

INTRODUCTION

Waste citrus activated sludge is the resulting product from the treatment of citrus processing plant wash water in a facility similiar to a municipal treatment plant. One of the major problems associated with this process is the production of excess activated sludge. The handling and disposal of sludge amounts to one of the larger operating costs of the system.

Previous pilot feeding trials with chicks, broilers, and laying hens (Jones *et al.*, 1975) were designed to gain basic information on practical levels of citrus sludge that could be utilized in feeds, and the level of toxicity and taste that might be passed to meat and eggs. These studies were undertaken to demonstrate the use of sludge as a feed ingredient to at least partially offset dewatering and disposal costs. Levels of 7.5 percent citrus sludge did not significantly affect performance of broilers or laying hens; however, above 10 percent citrus sludge, good body weights or egg production were not supported. The addition of sludge to the diet of broilers did not affect the palatability or texture of the meat. A significant improvement in egg yolk color was also associated with citrus sludge feeding, and the flavor of the yolk and the albumen were not affected.

It was felt from these studies that the recovery of activated sludge for use as an animal feed material was both technically feasible and economically attractive.

The current experiments were designed to further define this product as a feed ingredient and to explore the cause of depressed performance at higher levels of supplementation. Analytical determinations of nutrient content were the first necessary steps because the product tested in these studies differed from that used by Jones *et al.*, (1975) in both plant source and drying techniques. Trials were also designed to evaluate the biological availability of phosphorus, pigmenting value, and protein quality of citrus sludge in poultry feeds. The interaction of vitamin D_3 level and sludge in broiler feeds was also examined as a possible explanation for the depressed performance seen at higher levels of supplementation during earlier work, because Hurwitz (1957) had theorized that the iron and other heavy metals in sludge catalyzed vitamin D_3 destruction in feed.

As a basis for formulation, a proximate analysis including xanthophyll and carotene levels was conducted by the Feed Laboratory of the Florida Department of Agriculture and Consumer Services (Table 1).

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TABLE 1. PROXIMATE ANALYSIS OF CITRUS SLUDGE

Nutrient	Percent
Moisture	11.10
Crude Protein	22.35
Fat	7.40
Fiber	11.45
Ash	12.20
NFE	35.50
Calcium	1.60
Phosphorus	0.45
Xanthophyll	28.60mg/kg
Carotene	11.00mg/kg

To further define the nutritional profile of the product and look at the heavy metals present, determinations of the following factors were made by a commercial analytical laboratory (Law and Company; Atlanta, Georgia). The amino acid information is presented in Table 2 and the heavy metal determinations are summarized in Table 3.

TABLE 2. AMINO ACID PROFILE OF CITRUS SLUDGE

Amino Acid	Percent
Protein	24.2
Alanine	1.49
Arginine	0.83
Aspartic acid	2.05
Cystine	0.20
Glutamic acid	2.33
Glycine	1.14
Histidine	0.34
Isoleucine	1.03
Leucine	1.65
Lysine	1.02
Methionine	0.41
Phenylalanine	1.02
Proline	0.77
Serine	0.89
Threonine	1.03
Tryptophan	0.37
Tyrosine	0.57
Valine	1.22

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TABLE 3. TRACE MINERAL ANALYSIS OF CITRUS SLUDGE

Element	Content (ppm)[a]
Aluminum	2500
Cadmium	1
Chromium	100
Cobalt	3
Copper	260
Iron	4600
Lead	120
Magnesium	2100
Manganese	51
Mercury	0.5
Nickel	120
Potassium	7300
Selenium	<1
Sodium	5300
Zinc	430

[a]Dry-weight basis

Since citrus crops are often treated with pesticides that could carry through to the processing plant and waste water, the Florida State Pesticide Residue Laboratory was called upon to perform a routine screening analysis. Their results are indicated in Table 4. The residues were found to be well within normal levels and were not felt to be a potential hazard.

TABLE 4. PESTICIDE RESIDUE ANALYSIS OF CITRUS SLUDGE

Pesticide	Content (ppm)
Ethion	1.88
Dicofol	1.00
Screen - Chlorinated compounds	0
Screen - Phosphorus compounds	0

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SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The chemical and physical nature of citrus sludge made it a very difficult product to evaluate in feeding trials. The normal expectation of increased performance resulting from incremented levels of dietary inclusion were offset by a depressive agent in the cases of metabolizable energy determination and protein assay. Satisfactory conclusions could not be reached for either of these experimental objectives.

It had been postulated that vitamin D destruction by the high mineral levels contained in sludge might have been the cause of depressed performance; however a study with graded vitamin D_3 levels failed to confirm that suspicion.

Pigmentation trials with broilers and hens indicated that citrus sludge did have pigmentation value approximately equal to yellow corn in broiler skin and shanks, and egg yolks.

It is doubtful that a citrus sludge similiar to the one employed in these trials would be practically feasible in the commercial poultry industry because of poor nutrient content and, as yet unidentified, depressive agents. Further work is needed to improve the methods of concentrating, drying and handling the product to improve its nutrient content. Identification of the minerals that are reducing bird performance would also be necessary along with the determination of biological availability values.

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SECTION 3

SLUDGE PROCUREMENT AND DRYING

Approximately 1000 Kilograms of dried citrus sludge (11.1 percent moisture content) was obtained from Citrus World, Inc. of Lake Wales, Florida through the processing scheme discussed below.

Effluent was pumped from the sump of the first clarifier with a progressing cavity pump set to deliver approximately 132.5 liters/minute to a Sharples-Stokes batch-type basket centrifuge (model SP-5500). The centrifuge was equipped with a 60-horsepower motor powering a variable speed hydraulic drive assembly generating a centrifical force of 1300 G's. The unit was mounted on a trailer and equipped for full automatic operation on a uniform time cycle. An automatic skimmer attachment skimmed the cake to uniform thickness before it was plowed into the discharge basin. The resulting material was found to contain about 7 percent solids at this processing stage. To establish that the skimmer had been correctly set and had not taken off a portion of the nutrients, the check analysis shown in Table 5 was performed on fresh whole sludge. It was found that the dried product did contain the full complement of the elements found in the fresh product and that skimming had been effective.

TABLE 5. CHECK ANALYSIS OF SLUDGE EFFLUENT[a]

Nutrient	Percent (dry weight)
Total Nitrogen	4.32
Calcium	1.78
Phosphorus	0.48

[a]Analysis conducted by Environmental Testing Inc., Gainesville, Florida.

The sludge was placed into 206-liter drums and trucked to the Department of Poultry Science, Gainesville, Florida for additional drying. Drying was accomplished in a windowless room with a sloping concrete floor. A small commerical gas-fired crop dryer (250, 000 BTU/hr) controlled by a central thermostat set at 90°F provided and circulated dry heat. The sludge was stirred several times daily by hand with a large wooden rake.

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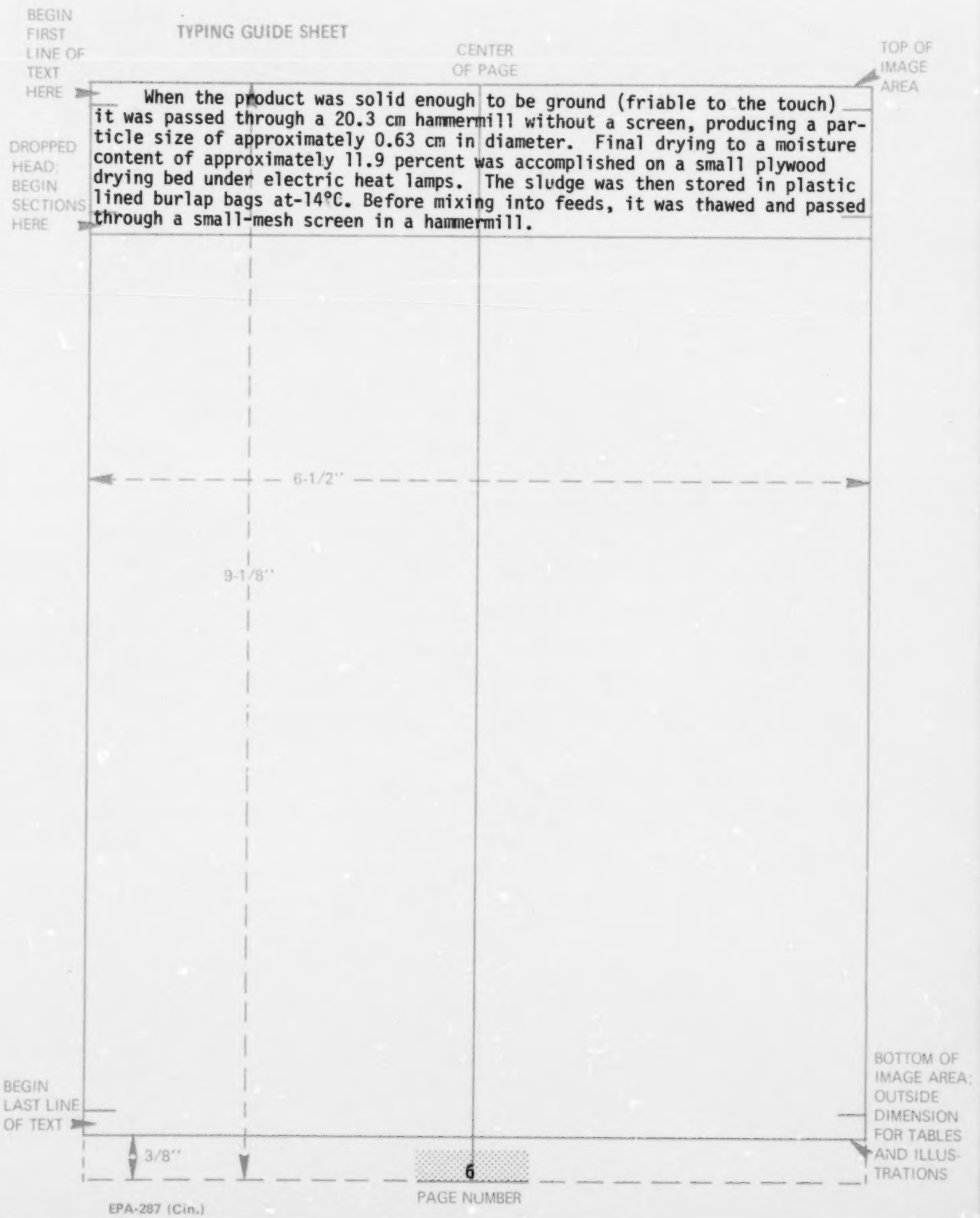
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When the product was solid enough to be ground (friable to the touch) it was passed through a 20.3 cm hammermill without a screen, producing a particle size of approximately 0.63 cm in diameter. Final drying to a moisture content of approximately 11.9 percent was accomplished on a small plywood drying bed under electric heat lamps. The sludge was then stored in plastic lined burlap bags at-14°C. Before mixing into feeds, it was thawed and passed through a small-mesh screen in a hammermill.
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SECTION 4

METABOLIZABLE ENERGY OF CITRUS SLUDGE

EXPERIMENTAL PROCEDURE

Seven groups of 10 male Cobb, color-sexed, broiler-type chicks were randomly assigned to electrically heated battery brooders with raised wire floors to prevent fecal recycling. They were fed a modified diet containing 40 percent sucrose (Table 6) according to the precedures of Hill *et al.* (1960). Chromic oxide, at a dietary level of 0.1 percent, was used as an index in order to eliminate the need for quantative collection of excreta and measurement of feed intake (Dansky and Hill, 1952).

TABLE 6. METABOLIZABLE ENERGY REFERENCE DIET

Ingredient	Percent
Sucrose	40.00
Yellow corn	12.30
Soybean meal (48.5%)	37.19
Alfalfa meal (17%)	3.50
Defluorinated Phosphate (18% P and 32% Ca)	2.35
Limestone	.96
Corn oil	2.44
DL-Methionine	.27
Microingredients[a]	.50
Iodized salt	.40

[a]Ingredients supplied per kilogram of diet: Vitamin A, 6600 IU; vitamin D_3, 2200 ICU; menadione dimethylpyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 499.4 mg; vitamin B_{12}, 22 ug. ethoxyquin, 00125%; manganese, 60 mg; iron, 50 mg; copper, 6 mg; cobalt, 0.0198 mg; zinc, 35 mg.

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At 14 days of age, each group was reduced to six birds and placed on a diet containing 0, 2, 4, 8 or 16 percent citrus sludge substituted for an equal amount of sucrose. During the 25th, 26th, and 27th days of the feeding period, representative samples of excreta (mixed feces and urine) were collected at 24-hour intervals and frozen for further processing. Samples of all diets were also taken for later analysis.

Feed and fecal samples were prepared for analysis by thawing, thoroughly blending the three daily subsamples into one container, drying to standard weight at 105°C for at least 24 hours, and finally grinding through a table-top Wiley mill with a 40-mesh screen.

The following measurements and/or determinations for metabolizable (ME) calculations were conducted at the end of the feeding period on both feed and excreta:

1. Moisture (percent dry matter)--by air oven at 105°C for 24 hours.

2. Gross energy expressed as Kcal/g of diet and excreta, determined by use of a Parr adiabatic bomb calorimeter according to methodology developed by Parr Instrument Company (1974) for foodstuffs.

3. Nitrogen content of diet and excreta determined by use of a Coleman model II nitrogen analyzer according to methodology suggested in the operation manual for solid samples (Perkin-Elmer, 1972).

4. Chromic oxide content of diet and excreta determined by use of a Perkin-Elmer model 290B atomic absorption spectrophotometer according to methodology suggested in Perkin-Elmer operation manual for chromium in feed and chromium in feces (Perkin-Elmer, 1976).

Final determination of the ME of citrus sludge was by the Hill and Anderson method developed at Cornell University in 1958. All methods used were as outlined by these authors with the exception of chromium and N determinations which were done by instrumentation rather than wet chemistry.

RESULTS AND DISCUSSION

The metabolizable energy content determined for alternative levels of citrus sludge addition is summarized in Table 7. The metabolizable energy of the sludge decreased numerically as the sludge substitution level in the diet was increased except for a slight unexplainable increase from the 6 to 8 percent level. It was noted during the feeding trials that at 16 percent sludge there was a laxative effect in the birds. This could have been caused by some unknown toxic constituent(s) in the sludge itself and may account for some of the decrease in utilization of sludge. Damron *et al.* (1976) noted a significant decrease in body weight and feed utilization in chicks fed dietary sludge levels of 10, 15 or 20 percent.

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TABLE 7. METABOLIZABLE ENERGY PER KILOGRAM OF CITRUS SLUDGE

Dietary Sludge Level (Percent)	Metabolizable energy (Kcal/kg)
2	3368
3	2411
4	1813
6	1360
8	1404
16	836

At the 2 percent level, the metabolizable energy was similar to that of yellow corn, but until the cause of the toxic effects of sludge is determined, an accurate and useful value for its metabolizable energy content in the chicken's diet cannot be developed because of the unknown factors involved.

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SECTION 5

CITRUS SLUDGE PROTEIN ASSAY

EXPERIMENTAL PROCEDURE

In two experiments, a graded series of protein levels (Table 8) were fed to day-old Cobb, color-sexed, broiler-type chicks for a three-week period in an attempt to assay the protein quality of citrus sludge. The birds were kept in electrically heated Petersime batteries with raised wire floors to prevent fecal recycling. Four replicate pens containing eight birds each received each of the dietary treatments. The 16 percent protein diet was used as a basal diet with the higher protein levels supplied either from corn-soybean or citrus sludge additions. A crude protein value of 24.2 percent and a metabolizable energy value of 2515 Kcal/kg were used for sludge when formulating these diets. The ME value was based on data collected previously in this study.

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Records for feed consumption and final body weights were kept along with mortality. All data were statistically analyzed by computerized S.A.S. procedures for analysis of variance and Duncan's multiple range test documented by Barr *et al.* (1976).

RESULTS AND DISCUSSION

A significant treatment X experiment interaction for body weight prevented data from the two experiments from being combined for presentation (Table 9). Statistical evaluation of other criteria indicated no such interaction, so values from individual experiments have been combined.

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TABLE 8. COMPOSITION OF CITRUS SLUDGE PROTEIN ASSAY DIETS

Ingredients	Percent crude protein supplied from--						
Corn-soy:	16	18	20	22	16	16	16
Sludge:	0	0	0	0	2	4	6
Yellow corn	75.26	68.54	61.94	54.76	61.60	47.62	33.90
Soybean meal (48.5%)	17.00	23.10	29.20	35.48	19.36	22.06	24.62
Citrus sludge	-----	-----	-----	-----	8.96	17.90	26.86
Limestone	1.01	0.98	0.96	0.92	0.98	0.90	0.88
Dicalcium phosphate (18% P and 32% Ca)	3.22	3.22	3.22	3.20	3.30	3.36	3.38
Iodized salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Microingredients[a]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Poultry fat	1.62	2.60	3.36	4.60	3.92	6.32	8.58
DL-methionine (98%)	0.33	0.28	0.20	0.14	0.36	0.38	0.38
Lysine (78.3%)	0.66	0.38	0.22	-----	0.62	0.56	0.50

[a]Ingredients supplied per kilogram of diet: Vitamin A, 6600 IU; vitamin D_3, 2200 ICU; menadione dimethyl-pyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 499.4 mg; vitamin B_{12}, 22 ug; ethoxyquin, 0.125%; manganese, 60 mg; iron, 50 mg; copper, 6 mg; cobalt, 0.0198 mg; zinc, 35 mg.

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TABLE 9. PROTEIN ASSAY CHICK PERFORMANCE*(3 WEEKS)

% Crude protein from		Body weight (g)		Feed intake**	Feed efficiency**
Corn-soy	Sludge	Exp 1	Exp 2	(g)	(g feed/g body wt.)
16	0	364^{e}	404^{d}	33.6^{c}	1.80^{c}
18	0	508^{b}	485^{b}	36.4^{ab}	1.53^{e}
20	0	549^{a}	536^{a}	36.8^{ab}	1.42^{f}
22	0	538^{a}	545^{a}	35.2^{bc}	1.36^{f}
16	2	479^{c}	458^{bc}	37.3^{ab}	1.70^{d}
16	4	410^{d}	430^{cd}	38.1^{a}	1.91^{b}
16	6	302^{f}	289^{e}	30.5^{d}	2.24^{a}

*Means without common letters are significantly different according to Duncan's multiple range test (<0.05).

**Values are a combination of two experiments.

In both experiments, body weight increased significantly up to the 20 percent protein level (corn-soy). When the additional protein (above 16 percent) came from sludge, the body weights of birds receiving 2 percent protein from sludge was significantly lower than that of a comparable corn-soy diet in experiment 1, but not in experiment 2. Similar comparisons at other sludge levels indicated significant reductions for sludge treated birds. In the 22 percent protein diet with 6 percent supplied as sludge, body weights were significantly below those of the 16 percent control groups in both experiments.

This negative effect of sludge at its highest level was also reflected in feed intake and feed efficiency. In the 18 and 20 percent diets, feed intake was not significantly different when the additional protein was from corn and soybeans rather than sludge. In the corn-soy diets, as the protein level increased, feed efficiency improved significantly. Just the opposite effect was observed with the sludge feeds. Mortality was not significant in any of the treatments.

At the lowest level of sludge addition, its protein availability appeared to be approximately 75 percent of that supplied by corn-soy. As the sludge level increased, its utilization decreased or was masked and a depressing effect occurred at the highest level of supplementation.

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SECTION 6

CITRUS SLUDGE-VITAMIN D_3 INTERACTION

EXPERIMENTAL PROCEDURE

In each of two experiments, a graded series of supplemental vitamin D_3 levels (200, 400, 800, and 1600 ICU) and sludge levels of 0, 5, 10, and 15 percent were fed in a corn-soy basal diet (Table 10) to day-old, broiler-type (Cobb) chicks for a three-week period. Four replicate pens containing eight birds each (four males and four females) received each dietary treatment. The chicks were grown in electrically heated Petersime battery brooders with raised wire floors. Records were kept for body weight, feed consumption, and mortality, as well as general observations on the condition of the birds.

Tibia ash values were determined for two birds of each sex for each pen. The entire bone (tibia) from the left leg of each bird was accurately weighed, placed in a tared crucible, ashed at 650°C for five hours, and then allowed to cool in a desiccator. The samples were then reweighed and ash values determined. Each sample was dissolved in approximately three ml of concentrated HCl, quantitatively transferred to a 250 ml volumetric flask, and brought up to volume with deionized water.

For the phosphorus determination, exactly one ml of each solution was transferred to another 250 ml volumetric flask and then about 25 ml of deionized water was added. To this solution was added 10 ml of ammonium molybdate solution; prepared by dissolving 12.5 g of ammonium molybdate in 100 ml of deionized water and adding this to 150 ml of 10 N sulfuric acid in a 500 ml volumetric flask. After the addition of the ammonium molybdate to the stock sample solution, four ml of 1-amino-2-naphthol-4 sulfonic acid (ANSA) reagent was added to each solution at one-minute intervals. The solutions were then brought up to volume, stoppered, and well shaken. After exactly 20 minutes transmittance values were obtained using a Perkin-Elmer UV-VIS spectrophotometer. These readings were applied to a standard curve and the amount of phosphorus present was calculated as a percentage of sample.

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TABLE 10. DIETS CONTAINING GRADED LEVELS OF CITRUS SLUDGE TO WHICH VARYING AMOUNTS OF VITAMIN D_3 WERE ADDED

Ingredients	Percent Sludge: 0	5	10	15
Yellow Corn	53.00	48.83	44.65	40.52
Soybean meal (49%)	38.00	36.34	34.67	32.80
Citrus Sludge	-----	5.00	10.00	15.00
Limestone	0.31	0.34	0.35	0.33
Dicalcium phosphate (18% P and 32% Ca)	3.26	3.22	3.26	3.30
Iodized salt	0.40	0.40	0.40	0.40
Microingredients[a]	0.50	0.50	0.50	0.50
Animal fat	4.42	5.22	5.95	6.81
DL-Methionine	0.11	0.15	0.19	0.24
L-lysine	-----	-----	0.02	0.10

[a]Ingredients supplied per kilogram of diet: Vitamin A, 6600 IU; vitamin D_3, 2200 ICU; menadione dimethylpyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 499.4 mg; vitamin B_{12}, 22ug; ethoxyquin, 0.0125% manganese, 60 mg; iron, 50 mg; copper, 6 mg; cobalt, 0.0198 mg; zinc, 35 mg.

For calcium determinations, 0.5 ml of the stock solution from each sample was placed in a 200 ml volumetric flask. To each of these was added 20 ml of stock lanthanum solution which was prepared by wetting 58.65 g of La_2O_3 with deionized water, then adding 250 ml of concentrated HCl very slowly until the material was dissolved and brought to 1000 ml volume with deionized water. After the addition of the lanthanum each flask was brought to volume with deionized water and agitated. These samples were read on a Perkin-Elmer atomic absorption spectrophotometer (Model 290B) and compared to a standard solution to determine the calcium percentage. All data obtained were statistically analyzed by computer using analysis of variance and Duncan's multiple range test (Barr *et al.*, 1976).

RESULTS AND DISCUSSION

There was no statistically significant (<0.05) interaction between sludge and vitamin D_3 for the data of Tables 12, 13 and 15. In Table 11, values for calcium were significantly different between experiments and, therefore, are reported separately. Significant treatment X experiment interactions for calcium in Table 12 were not present and the data have been combined for presentation.

In Table 11 there were no sitnificant differences between levels of sludge for values of calcium in experiment one or for phosphorus or ash in both experiments. There was an unexplainable increase in calcium at the 5 percent sludge level in experiment two. Feed efficiency (g feed/g body weight) decreased significantly at the 10 and 15 percent levels of sludge, with the 15 percent level being statistically higher than the 10 percent level.

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In Table 12, calcium, phosphorus, ash and feed efficiency values are expressed in terms of vitamin D_3 level and are based on the results of two experiments. Calcium was significantly lower at the 400 ICU level of D_3, while phosphorus was statistically equivalent at all levels of D_3. Ash values increased numerically as the level of D_3 increased, with the 200 and 1600 ICU levels being significantly different from each other. Feed efficiency values reflected no significant differences between level of D_3.

There was a significant interaction between sludge and vitamin D_3 in the data expressed in Tables 13 and 14. These data are summarized in two-way tables in an attempt to show this interaction. Table 13 expresses feed intake (g feed/bird day) on the basis of sludge and D_3 level. As the level of D_3 increased, feed intake increased, with the 1600 ICU level being significantly higher than the 200 and 400 ICU levels of D_3. Sludge had no significant effect on feed intake.

Body weights between experiments were found to be significantly different and are separated in Table 14. There was little variation in body weight in both experiments when expressed by levels of D_3, although the 400 ICU level weight was significantly lower than the 800 ICU level weight. However, when body weight was expressed on the basis of sludge level, there was a significant depression at the 15 percent level in both experiments. The highest numerical body weight was at the 5 percent sludge level in both experiments, and was significantly higher than the 0 percent level in experiment one.

Mortality for both experiments is shown in Table 15. There were no significant differences between levels of D_3. Based on sludge level, mortality was highest at the 5 percent level; however, no trend was evident.

In summary, the depressing effect that sludge had on body weight did not seem to be improved by the addition of vitamin D_3 to the diet of the chick. Therefore, the cause of the depression of body weight by sludge was probably not due to the destruction of D_3 by the high level of iron in sludge as was suspected by Hurwitz (1957). This lack of interaction was also expressed in ash values.

TABLE 11. CITRUS SLUDGE-VITAMIN D_3 INTERACTION CHICK PERFORMANCE (3 WEEKS)*

Sludge level (%)	Calcium (% of ash) Exp 1	Calcium (% of ash) Exp 2	Phosphorus** (% of ash)	Tibia ash** (%)	Feed efficiency** (g feed/g body wt)
0	37.08[a]	36.91[b]	17.37[a]	45.35[a]	1.48[c]
5	37.15[a]	41.11[a]	17.11[a]	45.38[a]	1.47[c]
10	36.99[a]	36.92[b]	17.26[a]	45.80[a]	1.55[b]
15	36.45[a]	37.13[b]	16.97[a]	45.43[a]	1.62[a]

*Means without common letter are significantly different according to Duncan's multiple range test (<0.05).

**Values are a combination of two experiments.

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TABLE 12. PERFORMANCE OF CHICKS ACCORDING TO VITAMIN D_3 LEVEL FED (3 WEEKS)*

D_3 level (ICU)	Calcium** (% of ash)	Phosphorus** (% of ash)	Tibia Ash** (%)	Feed efficiency** (g feed/g body wt)
200	38.06[a]	17.25[a]	45.19[b]	1.52[a]
400	36.05[b]	17.04[a]	45.43[ab]	1.53[a]
800	38.10[a]	17.37[a]	45.54[ab]	1.52[a]
1600	37.65[a]	17.05[a]	45.80[a]	1.55[a]

*Means without common letters are significantly different according to Duncan's multiple range test (<0.05).

**Values are a combination of two experiments.

TABLE 13. DAILY FEED INTAKE OF CHICKS THAT RECEIVED VARIOUS LEVELS OF CITRUS SLUDGE AND VITAMIN D_3* (EXPERIMENTS 1 AND 2)

D_3 level (ICU)	Sludge level (% of diet)				Mean
	0	5	10	15	
200	36.99	37.26	37.11	36.81	37.04[b]
400	37.64	36.43	37.30	34.20	36.39[b]
800	37.73	37.60	37.69	37.84	37.71[ab]
1600	38.13	37.76	38.55	40.39	38.71[a]
Mean	37.62[a]	37.26[a]	37.66[a]	37.31[a]	

*Means without common letters are significantly different according to Duncan's multiple range test (<0.05).

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TABLE 14. TWO-WAY BODY WEIGHT RESULTS OF CHICKS THAT RECEIVED VARIOUS LEVELS OF CITRUS SLUDGE AND VITAMIN D_3*

D_3 level (ICU)	Sludge level (% of diet) 0	5	10	15	Mean
			Experiment 1		
200	541	532	501	492	517^{ab}
400	527	574	520	410	508^{b}
800	531	566	536	484	529^{a}
1600	533	541	542	480	524^{ab}
Mean	533^{b}	553^{a}	524^{b}	466^{c}	
			Experiment 2		
200	536	535	495	490	514^{ab}
400	539	535	500	471	511^{b}
800	537	545	533	499	528^{a}
1600	509	538	520	479	512^{b}
Mean	530^{ab}	538^{a}	512^{b}	485^{c}	

*Means without common letters are significantly different according to Duncan's multiple range test (<0.05).

TABLE 15. MORTALITY OF CHICKS THAT RECEIVED VARIOUS LEVELS OF CITRUS SLUDGE AND VITAMIN D_3*

Sludge level (%)	Mortality (%)	Vitamin D_3 Level (ICU)	Mortality (%)
0	1.56^{ab}	200	1.17^{a}
5	3.90^{a}	400	1.17^{a}
10	.78^{b}	800	3.90^{a}
15	2.73^{ab}	1600	2.73^{a}

*Means without common letters are significantly different according to Duncan's multiple range test (<0.05).

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SECTION 7

PHOSPHORUS ASSAY OF CITRUS SLUDGE

EXPERIMENTAL PROCEDURE

Duplicate experiments were conducted to evaluate the biological availability of the phosphorous contained in citrus sludge. A modified corn-soy basal diet (Table 16) calculated to contain 0.34 percent phosphorous, 0.095 percent calcium, 21 percent protein, 0.86 percent sulfur containing amino acids, 1.25 percent lysine, and 3080 kilocalories of metabolizable energy per kilogram, was employed with the variable ingredients consisting of amounts of citrus sludge, monosodium phosphate, calcium carbonate, sucrose, and filler necessary to provide the various dietary treatments as shown in Table 17. A level of 0.05 percent supplemental phosphorus was provided from sludge along with three total calcium levels for maximum expression of availability. Standard diets for comparison contained either 0.025 or 0.05 percent phosphorus from sodium acid phosphate, each with three total calcium levels (Harms *et al*, 1965).

TABLE 16. PHOSPHORUS ASSAY BASAL DIET

Ingredients	Percent
Yellow corn	49.77
Soybean meal (49%)	34.03
Animal fat	2.86
Iodized Salt	.40
Microingredients[a]	.50
DL - Methionine	.18
L - Lysine	.05
Variable[b]	12.21

[a] Supplied the following activities per kilogram of diet: Vitamin A, 6600 I.U. vitamin D_3 2200 I.C.U.; menadione dimethyl-pyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid 13.2 mg; niacin, 39.6 mg; choline chloride, 499.4 mg; vitamin B_{12}, 22 ug; ethoxyquin, 0.0125%; manganese, 60 mg; iron, 50 mg; copper, 6 mg; cobalt, 0.198 mg; iodine, 1.1 mg; zinc, 35 mg.

[b] Included the amounts of citrus sludge, monosodium phosphate, calcium carbonate, sucrose and filler necessary to provide the various dietary treatments.

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TABLE 17. RESPONSE OF 3-WEEK OLD CHICKS TO THE FEEDING OF VARIOUS LEVELS OF CALCIUM AND PHOSPHORUS FROM CITRUS SLUDGE AND STANDARD SOURCES*

Treatment (%) Supp. P	Total Ca	Body Wt. (grams)	Daily Feed (grams)	Tibia Ash (%)
0.05 (mono)	.43	470 a	33.2 abc	34.20 c
0.05 (mono)	.50	486 a	34.80 ab	36.44 ab
0.05 (mono)	.57	494 a	35.30 a	37.24 a
0.025 (mono)	.41	469 a	32.66 abc	34.89 bc
0.025 (mono)	.47	478 a	34.46 ab	35.23 bc
0.025 (mono)	.53	486 a	34.53 ab	36.98 a
0.05 (Sludge)	.52	409 b	30.75 c	35.57 abc
0.05 (Sludge)	.58	423 b	31.89 bc	35.90 abc
0.05 (Sludge)	.64	421 b	32.62 abc	35.15 bc

*Means represent the average of two experiments and differ significantly according to Duncan's multiple range test if they have superscripts without common letters.

Four replicate groups, each containing four male and four female day-old broiler-type chicks, received each dietary treatment for a three-week feeding period in battery brooders with raised wire floors. The birds were sexed, wing-banded; and randomly assigned to treatment pens at one day of age. Feed and water were provided *ad lib* along with continuous lighting.

On the final day of each experiment, the birds were separated and weighed according to sex, and two males and two females from each replicate group were sacrified for tibia ash determinations. The A.O.A.C. (1970) procedure for vitamin D_3 determination was followed in ashing the bones. Data were subjected to computerized analysis of variance and Duncan's multiple range test procedures through the Statistical Analysis System developed by North Carolina State University (Barr, *et al.*, 1976).

RESULTS AND DISCUSSION:

In terms of body weight, the addition of either 0.025 or 0.05 percent supplemental phosphorus from monosodium phosphate had no significant influence. (Table 17) However, increasing calcium levels within a phosphorus level supported some numerical improvement in body weights. All sludge treatments (0.05 percent supplemental phosphorous) had weights significantly less than treatments receiving supplemental monosodium phosphate diets. The calcium level effect was, again, evident but not significant. If one uses the best growth results from monosodium phosphate (494 g) and sludge (423 g) as a basis of calculation, sludge diets supported approximately 85 percent of the body weight with some of the improvements due to calcium level being significant. Calcium level did not affect tibia ash of sludge treatments. Ash values of two citrus sludge treatments (0.52 and 0.58 percent Ca) did not differ significantly from any of the 0.05 percent monosodium phosphate treatments. The highest of the sludge treatment tibia ash values (35.9 percent) was 96 percent of that supported by the best monocalcium phosphate treatment

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(0.05 percent P, 57 percent Ca).

The failure of sludge treatments to produce better results may have been due to the reduced feed intake associated with those treatments. This reduction was felt to be due to the large amount of sludge required to add phosphorus to the test. The study was originally designed using phosphorus values from another sludge; however, the sludge used in these tests did not have nearly as high a level. For this reason, the levels of inclusion were very low and a variety of additions were not possible.

Mortality was not a factor since only two deaths were recorded in different treatments.

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SECTION 8

EVALUATION OF CITRUS SLUDGE AS AN EFFECTIVE EGG YOLK PIGMENTING INGREDIENT

The high costs of feed ingredients and the problem of xanthophyll variation among feedstuffs has resulted in searches for alternative xanthophyll ingredient sources. Many exotic xanthophyll ingredient sources have been examined including paprika, dried algae, and broccoli leaf meal. The purpose of this experiment was to evaluate the egg yolk pigmenting efficiency of waste activated citrus sludge.

EXPERIMENTAL PROCEDURE

A commercial strain of White Leghorn hens was fed a white corn, basal, xanthophyll-free diet for 15 days to deplete the egg yolks of all pigment. In each of the two trials, there were two replicate groups of five individually caged hens per treatment and nine treatment groups, each fed a different experimental diet. These diets included a white corn basal diet and feeds containing 2.5, 5.0, 7.5, or 10.0 percent citrus sludge (Table 18). The xanthophyll content of citrus sludge was determined by the State Feed Laboratory of the Florida Department of Agricultural and Consumer Services. Four yellow corn diets were formulated to contain the same amount of xanthophyll as each of the citrus sludge diets, respectively. After 21 days on the experimental diet, eggs were collected and held (at 12.8°C and 85 percent RH) for yolk pigmentation analysis.

Eggs obtained from these diets were broken and the contents hand separated. This was accomplished by a gentle swirling motion while the liquid egg components were loosely cupped in the palm of the hand. After most of the albumen had been removed the yolk was allowed to roll across a dampened paper towel to remove the chalazae. The vitelline membrane was also removed to prevent interference during sample reading.

Blending of the egg yolk was accomplished by use of a mixing spatula. Both hand separation and hand blending were used in lieu of mechanical separation and blending due to the desirability of avoiding chalazae residues and bubbles.

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TABLE 18. COMPOSITION OF DIET FOR EXPERIMENTS 1 AND 2

Ingredients	White corn	Yellow corn basal (3.74 mg xanthophyll/Kg)[a] Percent	Citrus sludge basal (3.74 mg xanthophyll/kg)[b] percent
White corn	70.02	37.35	61.00
Yellow corn (15.7 mg xanthophyll/kg)	-----	-----	-----
Citrus sludge[c]	-----	-----	-----
Soybean meal (50% protein)	19.00	19.00	16.02
Limestone	6.88	6.88	6.74
Dicalcium phosphate (18.5% P; 22% Ca)	2.25	2.25	2.48
Iodized salt	0.35	0.35	0.35
Microingredient mix[d]	0.50	0.50	0.50
Animal fat	-----	-----	-----
DL-Methionine	-----	-----	-----
Sand	1.00	1.00	0.33

[a]This diet was equivalent in xanthophyll level to the citrus sludge basal (10 .0%). To formulate diets with xanthophyll levels equivalent to the 2.5, 5.0, and 7.5% citrus sludge diet, the following blends were used:

Yellow corn ('2.5') = white corn basal (75%) + yellow corn basal (25%)
Yellow corn ('5.0') = white corn basal (50%) + yellow corn basal (50%)
Yellow corn ('7.5') = white corn basal (25%) + yellow corn basal (75%)

[b]To formulate 2.5, 5.0, and 7.5% citrus sludge diets, the following blends were used:
Citrus sludge (2.5%) = white corn basal (75%) + citrus sludge basal (25%)
Citrus sludge (5.0%) = white corn basal (50%) + citrus sludge basal (50%)
Citrus sludge (7.5%) = white corn basal (25%) + citrus sludge basal (75%)

[c]22.4% protein, 0.45% P, 1.6% Ca, ME = 800 Kcal.

[d]Ingredients supplied per kilogram of diet: Vitamin A, 6600 IU; vitamin D_3, 2200 ICU; menadione dimethylpyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 499.4 mg; vitamin B_{12}, 22 ug; ethoxyquin, 0.0125%; manganese, 60 mg; iron, 50 mg; copper, 6 mg; cobalt, 0.0198 mg; zinc, 35 mg.

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The samples obtained from pooled egg yolks were placed in lucite sample holders and held in place over the aperature of a IDL COLOR-EYE.^{R} Readings for X, X^{1}, Y and Z were then taken from the COLOR-EYE. From these readings luminosity (^{Y}CIE), excitation purity (EP), and dominant wavelength (DWL) were calculated using the AMDAHL 470 computer and a program prepared by Fry and Damron (1971).

This procedure produced two samples per treatment group. All comparisons were made between each citrus sludge diet and the corresponding yellow corn diet containing an equivalent xanthophyll level.

The data were analyzed using analysis of variance procedures from the Statistical Analysis Systems programs (Barr *et al.*, 1976) and significant differences determined by the Duncan's multiple range test (Steel and Torrie, 1960). Since there was an experiment X treatment interaction, the two trials were analyzed separately.

RESULTS AND DISCUSSION

Experiment 1

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The small variation in feed efficiencies (Table 19) indicated that the xanthophyll intake per dozen eggs was approximately the same. When comparing the DWL values (Table 20), a significant difference was found between the 2.5 percent citrus sludge and the corresponding xanthophyll level yellow corn diet; the yellow corn diet imparted a more orange hue to the egg yolks. However, when comparing the 5.0, 7.5 and 10.0 percent citrus sludge diets to the appropriate yellow corn diets, no significant differences were found in DWL values. Luminosity (LUM) values were also not different when citrus sludge and yellow corn were fed at the same xanthophyll levels. Excitation purity (EP) values were significantly higher for yellow corn compared to citrus sludge at equivalent xanthophyll levels. This indicated that the yellow corn diets resulted in more pigment being present in the egg yolks than the citrus sludge diets. Increasing dietary citrus sludge resulted in increases in the DWL and EP values, that is, more pigment and more orange color is present in the yolks produced by hens fed a higher xanthophyll level. Visual scores also increased as dietary xanthophyll level increased. The DWL and EP values also increased as the xanthophyll level supplied by yellow corn increased. In Experiment 1, the xanthophylls in citrus sludge imparted the same hue (DWL) as yellow corn. Tha xanthophylls in yellow corn, however, appeared to be more biologically available (EP).

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TABLE 19. PERCENT PRODUCTION (HEN-DAY), FEED EFFICIENCY, AND FEED CONSUMPTION FOR HENS FED CITRUS SLUDGE OR YELLOW CORN AT EQUIVALENT XANTHOPHYLL LEVELS IN EXPERIMENT 1 AND 2

Treatment	Experiment 1 Percent egg production (hen-day)	Kg feed / Dz eggs	G feed / Hen/day
White corn	69.1 ± 0.45*	1.60 ± 0.04	92.00 ± 3.24
Citrus sludge			
2.5%	67.6 ± 1.90	1.57 ± 0.09	88.20 ± 8.10
5.0%	63.4 ± 0.45	1.81 ± 0.07	95.76 ± 2.70
7.5%	78.2 ± 1.10	1.64 ± 0.03	103.86 ± 4.33
10.0%	72.4 ± 0.00	1.75 ± 0.04	100.10 ± 0.04
Yellow corn			
'2.5'**	76.2 ± 0.00	1.52 ± 0.02	96.86 ± 1.62
'5.0'	68.6 ± 3.95	1.72 ± 0.02	92.57 ± 1.62
'7.5'	68.4 ± 8.75	1.78 ± 0.18	100.18 ± 3.75
'10.0'	70.4 ± 0.95	1.63 ± 0.01	96.34 ± 1.10

Treatment	Experiment 2 Percent egg production (hen-day)	Kg feed / Dz eggs	G feed / Hen/day
White corn	52.7 ± 1.80*	2.22 ± 0.20	91.41 ± 5.68
Citrus sludge			
2.5%	70.5 ± 0.45	1.76 ± 0.02	105.50 ± 4.50
5.0%	55.9 ± 8.60	2.10 ± 0.30	95.39 ± 2.55
7.5%	65.9 ± 4.10	1.94 ± 0.10	106.18 ± 1.00
10.0%	65.0 ± 5.00	1.87 ± 0.13	100.50 ± 0.50
Yellow corn			
'2.5'**	66.4 ± 1.85	1.79 ± 0.11	99.46 ± 2.55
'5.0'	68.6 ± 4.10	1.79 ± 0.11	102.09 ± 0.00
'7.5'	65.9 ± 1.40	1.83 ± 0.04	100.50 ± 0.50
'10.0'	61.4 ± 0.45	1.89 ± 0.11	96.91 ± 6.18

*Standard error of the mean.
**The single quote marks (') indicate that the diet contained a xanthophyll level equivalent to the corresponding citrus sludge diet.

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TABLE 20. VISUAL SCORES, DWL, EP, AND LUM VALUES FOR EGG YOLKS FROM HENS FED CITRUS SLUDGE OR YELLOW CORN DIETS AT EQUIVALENT XANTHOPHYLL LEVELS

Experiment 1

Treatment	Visual score	DWL (nm)**	EP (%)**	LUM (%)**
White corn	1.1 ± 0.08*	572.3^{a}	34.65^{a}	38.59^{cd}
Citrus sludge				
2.5%	1.7 ± 0.26	573.0^{b}	40.38^{b}	39.96^{d}
5.0%	2.5 ± 0.34	573.9^{d}	47.18^{c}	37.74^{cd}
7.5%	3.2 ± 0.44	574.6^{fg}	54.66^{de}	35.75^{abc}
10.0%	3.7 ± 0.38	574.9^{g}	58.80^{ef}	34.34^{ab}
Yellow corn				
'2.5'***	1.8 ± 0.17	573.5^{c}	50.29^{cd}	38.57^{cd}
'5.0'	3.0 ± 0.21	574.2^{de}	62.01^{fg}	36.50^{abc}
'7.5'	4.8 ± 0.21	574.4^{ef}	66.43^{g}	33.75^{a}
'10.0'	5.2 ± 0.27	574.9^{g}	73.35^{h}	34.70^{ab}

Experiment 2

Treatment	Visual score	DWL (nm)	EP (%)	LUM (%)
White corn	1.1 ± 0.08*	571.7^{a}	33.15^{a}	40.23^{c}
Citrus sludge				
2.5%	2.4 ± 0.26	572.8^{a}	41.94^{b}	39.04^{bc}
5.0%	3.6 ± 0.23	573.5^{a}	50.03^{c}	36.98^{abc}
7.5%	3.8 ± 0.34	574.0^{a}	53.49^{d}	35.69^{abc}
10.0%	4.2 ± 0.36	574.2^{a}	55.80^{d}	35.48^{abc}
Yellow corn				
'2.5'***	4.0 ± 0.23	573.4^{a}	56.86^{d}	37.69^{bc}
'5.0'	5.3 ± 0.28	574.0^{a}	68.92^{e}	36.86^{abc}
'7.5'	7.0 ± 0.17	574.9^{a}	78.38^{f}	33.88^{ab}
'10.0'	7.9 ± 0.15	575.3^{a}	80.84^{g}	32.05^{a}

*Standard error of the mean
**Means within the same column with different superscripts are significantly different (<0.05).
***The single quote marks (') indicate that the diet contains a xanthophyll level equivalent to the corresponding citrus sludge diet.

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Experiment 2

As in experiment 1, the xanthophyll intake per dozen eggs was approximately the same when comparing each citrus sludge diet with the corresponding yellow corn diet containing the equivalent xanthophyll level (Table 19). There were no differences in DWL or LUM values when comparing each citrus sludge diet to the appropriate yellow corn diet (Table 20); however, there were significant differences in the EP values (Table 20) when comparing citrus sludge and yellow corn at equivalent xanthophyll levels. This suggests that the xanthophylls in yellow corn were more available than those in citrus sludge. Visual scores were also higher for the yolks from hens fed yellow corn at each of the xanthophyll levels. Again, there was an increase in DWL, EP, and visual scores with increasing dietary xanthophyll level whether yellow corn or citrus sludge was fed.

These data indicated that egg yolks produced by hens fed citrus sludge were the same color (similar DWL and LUM values) as those produced by hens fed yellow corn at equivalent xanthophyll levels, although the egg yolks produced by the hens fed citrus sludge do not have as much pigment (EP value) as those produced by hens fed yellow corn. If eggs are produced for home table egg consumption, citrus sludge would be a suitable pigmenting agent. If the eggs are produced for further processed products such as mayonnaise and noodles, however, citrus sludge would not be as suitable as yellow corn, since there is less pigment in the yolks and this pigment would be diluted out and result in processed egg products with less than the desirable color.

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SECTION 9

EVALUATION OF CITRUS SLUDGE AS AN EFFECTIVE BROILER SKIN PIGMENTING INGREDIENT

A pilot study has shown waste citrus activated sludge to be a suitable ingredient in broiler diets (Eldred *et al.*, 1975). This study was designed to further examine the influence of citrus sludge on broiler pigmentation.

EXPERIMENTAL PROCEDURE

In each of two experiments equal numbers (nine) of day-old male and female, Cobb color-sexed chicks were randomly distributed into pens (2.32 m^2) providing a density of 0.129 m^2/bird. All birds were brooded on peanut hull litter over concrete flooring with infrared lights used for heat. From day one to the end of the 4th week, the birds were fed a white corn, xanthophyll free, starter diet. From the beginning of the 5th week, two replicate pens were fed one of nine experimental diets (Table 21). At 56 days of age, five males and five females from each pen were killed by cervical dislocation. The broilers were scalded at 51.7°C for 90 seconds after the right shank had been removed at the hock. An area approximately 5 cm x 5 cm square on the right thigh was hand plucked and the exposed skin with feather tracts was excised. The skin samples and shanks were packaged and frozen until analyzed for pigmentation using the IDL COLOR-EYE^R as described by Fry *et al.*,(1969). The COLOR-EYE^R values were changed to DWL (dominant wavelength), EP (excitation purity) and LUM (luminosity) values using the computer program developed by Fry and Damron (1971). The DWL value indicates the actual hue of the majority of the reflected light from the samples. The EP value indicates the intensity or amount of the pigment present in the sample at that DWL value. The LUM value indicates the brightness or darkness of the sample. All comparisons were made between each citrus sludge diet and the corresponding yellow corn diet containing the same amount of xanthophyll, that is, the 2.5 percent citrus sludge diet is compared to the yellow corn diet which had the equivalent xanthophyll level.

The data were analyzed using standard error of the mean, analyses of variance, and Duncan's multiple range test (Steel and Torrie, 1960). Because of an experiment X treatment interaction, the two experiments were analyzed separately.

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RESULTS AND DISCUSSION

Experiment 1

When the citrus sludge diets were compared with the corresponding yellow corn diets, there were only small variations in feed efficiency (Table 22), indicating that xanthophyll intake per kilogram body weight was similar for all treatments.

The DWL values of the shanks (Table 23) were not different when comparing 5.0, 7.5, and 10.0 percent citrus sludge diets with the corresponding yellow corn diets. When comparing the 2.5 percent sludge diet with the corresponding yellow corn diet there was, however, a significant difference in the DWL values. The yellow corn diet had imparted more orange hue to the shanks than the citrus sludge diet.

Citrus sludge or yellow corn in the diet resulted in more pigment present in the shanks than the control or white corn diet, indicated by EP values (Table 23). There were significant increases in EP values with increases in xanthophyll levels supplied by yellow corn. The yellow corn diets also resulted in more pigment present in the shanks than the citrus sludge diets (EP values). When comparing each citrus sludge diet to the corresponding yellow corn diet containing the equivalent xanthophyll level, there were significant increases in EP values for the shanks of the broilers fed the yellow corn. Although there was a significant difference in the LUM values when comparing the 5.0 percent citrus sludge diet to the corresponding yellow corn diet, there were no other differences in the LUM values for the shanks indicating that all the shanks were approximately the same brightness.

The hue (DWL values - Table 23) of the skin samples from the broilers fed 2.5, 5.0, 7.5, and 10.0 percent citrus sludge did not differ from that of the skin from broilers fed the corresponding yellow corn diets containing equivalent xanthophyll levels. A comparison of skin EP values (Table 23), however, indicated a significant difference between the 7.5 and 10.0 percent citrus sludge diets and their xanthophyll equivalent yellow corn diets, with the yellow corn imparting more pigment to the skins. Trends, although not significant, also suggest this to be true when comparing the EP values for the skin of the broilers fed the 2.5 and 5.0 percent citrus sludge diets to those of the broilers fed the yellow corn diet containing the equivalent xanthophyll level. The LUM values for citrus sludge diets were not significantly different from corresponding yellow corn diets. In experiment 1, feeding citrus sludge resulted in the same hue and darkness in both broiler shank and skin samples as feeding yellow corn at comparable xanthophyll levels. The EP values, however, indicated that the xanthophylls in yellow corn were slightly more available than those in the citrus sludge.

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Experiment 2

As in experiment 1, the feed efficiencies (Table 24) did not differ when comparing the citrus sludge diets to their comparable yellow corn diets. The DWL, EP, and LUM values for the shanks are shown in Table 25. Although there is a significant difference in shank DWL values when comparing the 10.0 percent citrus sludge diet with the equivalent xanthophyll level yellow corn diet, trends suggest, as in experiment 1, that there is no difference in hue obtained from feeding citrus sludge when compared to feeding yellow corn at the same xanthophyll level.

Shank EP values also followed the pattern found in Experiment 1. The EP values for the shanks from the broilers fed the yellow corn diet were significantly higher than those from the broilers fed the corresponding citrus sludge diets. In experiment 2, however, there were increases in EP values with an increase in dietary citrus sludge. Luminosity (LUM) values, as in experiment 1, did not differ when comparing the corresponding citrus sludge and yellow corn diets.

The DWL, EP, and LUM values for the skin samples are shown in Table 25. Again, there were no differences in the DWL of the skin samples when comparing citrus sludge and yellow corn fed at equivalent xanthophyll levels. The only significant difference in LUM values occurred when comparing the 2.5 percent citrus sludge diet to the corresponding yellow corn diet. The EP values for the 2.5 percent citrus sludge and the corresponding yellow corn diet did not differ. When comparing the EP values for the other citrus sludge diets, all were significantly different from the corresponding yellow corn diets; with the yellow corn diets again imparting more pigment to the skin samples. As in experiment 1, feeding citrus sludge to broilers resulted in imparting the same hue and darkness to shank and skin samples as feeding yellow corn at comparable xanthophyll levels. The experiment treatment interaction appeared to be due to the difference in the relationship of the EP values with increasing dietary citrus sludge levels. In Experiment 2, increasing citrus sludge in the diet increased xanthophyll content in the shanks of the broilers. This relationship was not present in experiment 1. In both experiments, however, it appeared that the xanthophylls in yellow corn are more available than those in citrus sludge as indicated by the EP values.

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TABLE 21. COMPOSITION OF DIETS IN EXPERIMENTS 1 AND 2 (PERCENT)

Ingredient	Citrus sludge (10.0%) (3.74 mg xanth/kg)	Citrus sludge (7.5%) (2.81 mg xanth/kg)	Citrus sludge (2.5%) (0.93 mg xanth/kg)	Citrus sludge (2.5%) (0.93 mg xanth/kg)	Yellow corn basal[a]	White corn basal
White corn	45.76	48.06	50.36	52.66	-----	54.94
Yellow corn	-----	-----	-----	-----	54.94	-----
Citrus sludge[b]	10.00	7.50	5.00	2.50	-----	-----
Soybean meal (50% protein)	35.46	36.25	37.00	37.82	38.63	38.63
Limestone	0.67	0.80	0.94	1.08	1.22	1.22
Dicalcium phosphate (18.5% P; 22% Ca)	2.04	2.04	2.04	2.04	2.05	2.05
Iodized salt	0.40	0.40	0.40	0.40	0.40	0.40
Microingredient mix[c]	0.50	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.18	0.16	0.16	0.12	0.09	0.09
Animal fat	4.94	4.24	3.55	2.83	2.12	2.12
Amprol	0.05	0.05	0.05	0.50	0.05	0.05

[a]To mix yellow corn diets containing xanthophyll levels equivalent to the citrus sludge diets, the following blends were used:

Yellow corn ('10.0') = yellow corn basal (42.61%) + white corn basal (57.39%)
Yellow corn ('7.5') = yellow corn basal (31.95%) + white corn basal (68.05%)
Yellow corn ('5.0') = yellow corn basal (21.30%) + white corn basal (78.70%)
Yellow corn ('2.5') = yellow corn basal (10.65%) + white corn basal (89.35%)

[b]22.4% protein, 1.6% Ca, 0.45% P, ME = 800 Kcal.

[c]Ingredients supplied per kilogram of diet: Vitamin A, 6600 IU; vitamin D_3, 2200 ICU; menadione dimethylpyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 499.4 mg; vitamin B_{12}, 22 ug; ethoxyquin, 0.0125%; manganese, 60 mg; iron, 50 mg; copper, 6 mg; cobalt, 0.0198 mg; zinc, 35 mg.

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TABLE 22. BODY WEIGHTS AND FEED EFFICIENCIES FOR BROILERS FED VARIOUS CITRUS SLUDGE AND YELLOW CORN DIETS FROM 4-8 WEEKS AT EQUIVALENT XANTHOPHYLL LEVELS - EXPERIMENT 1

Treatment	Sex	Body weights[a]	Feed efficiency[b]
White corn	Male	1213.00 ± 39.67[c]	
	Female	946.93 ± 17.52	
	Mean	1080.60 ± 46.93	2.61 ± 0.01
Citrus sludge 2.5%	Male	1234.84 ± 22.86	
	Female	923.79 ± 25.85	
	Mean	1079.32 ± 24.15	2.60 ± 0.04
Citrus sludge 5.0%	Male	1188.44 ± 30.24	
	Female	925.00 ± 25.05	
6-1/2"	Mean	1056.72 ± 37.66	2.73 ± 0.04
Citrus sludge 10.0%	Male	1242.13 ± 35.36	
	Female	954.17 ± 21.35	
	Mean	1098.15 ± 28.35	2.63 ± 0.02
9-1/8"			
Yellow corn '2.5'	Male	1184.95 ± 53.43	
	Female	941.10 ± 16.95	
	Mean	1063.03 ± 35.01	2.68 ± 0.09
Yellow corn '5.0'	Male	1219.67 ± 58.45	
	Female	933.89 ± 35.29	
	Mean	1076.79 ± 45.46	2.60 ± 0.06
Yellow corn '7.5'	Male	1229.74 ± 30.50	
	Female	932.32 ± 33.72	
	Mean	1081.03 ± 32.10	2.60 ± 0.05
Yellow corn '10.0'	Male	1285.19 ± 20.87	
	Female	945.0 ± 6.94	
	Mean	1115.09 ± 11.74	2.56 ± 0.03

[a]Weight gained from the 4-9 week period.

[b]Feed efficiency for the 4-8 week period.

[c]Standard error of the mean.

[d]The single quote marks (') indicate that the diet contains a xanthophyll level equivalent to the corresponding citrus sludge diet.

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TABLE 23. DWL, EP, AND LUM VALUES FOR SHANK AND SKIN SAMPLES FROM BROILERS FED CITRUS SLUDGE OR YELLOW CORN DIETS AT EQUIVALENT XANTHOPHYLL LEVELS IN EXPERIMENT 1

Treatment	Shanks			Skin		
	DWL (nm)[a]	EP (%)	LUM (%)	DWL (nm)	EP (%)	LUM (%)
White corn	579.9^{d}	20.48^{a}	62.21^{ab}	582.8^{b}	18.80^{a}	59.46^{ab}
Citrus sludge						
2.5%	578.0^{a}	22.30^{b}	62.13^{ab}	582.9^{b}	18.92^{a}	60.34^{ab}
5.0%	579.8^{cd}	23.20^{bc}	59.83^{a}	582.4^{ab}	19.35^{a}	59.51^{ab}
7.5%	579.1^{abcd}	22.09^{b}	62.06^{ab}	581.6^{ab}	18.70^{a}	60.98^{b}
10.0%	579.6^{bcd}	22.66^{b}	59.74^{a}	582.2^{ab}	19.75^{a}	60.11^{ab}
Yellow corn						
'2.5'	579.5^{bcd}	24.11^{c}	61.39^{a}	582.0^{ab}	19.02^{a}	61.92^{b}
'5.0'	578.6^{abcd}	26.93^{d}	62.55^{b}	582.1^{ab}	20.04^{a}	60.61^{ab}
'7.5'	578.3^{ab}	31.33^{e}	61.83^{ab}	580.5^{a}	21.27^{b}	61.44^{b}
'10.0'	578.5^{abc}	34.74^{f}	60.33^{ab}	582.1^{ab}	22.27^{b}	58.28^{a}

[a]Means within each column with different superscripts are significantly different (<0.05).
[b]The single quote marks (') indicate that the diet contained a xanthophyll level equivalent to the corresponding citrus sludge diet.

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TABLE 24. BODY WEIGHTS AND FEED EFFICIENCIES FOR BROILERS FED VARIOUS CITRUS SLUDGE AND YELLOW CORN DIETS FROM 4-8 WEEKS AT EQUIVALENT XANTHOPHYLL LEVELS - EXPERIMENT 2

Treatment	Sex	Experiment 2 Body weights[a]	Feed efficiency[b]
White corn	Male	1572.79 ± 46.05[c]	
	Female	1209.26 ± 21.83	
	Mean	1391.56 ± 32.26	1.94 ± 0.07[c]
Citrus sludge 2.5%	Male	1502.00 ± 58.10	
	Female	1190.25 ± 14.50	
	Mean	1346.13 ± 24.83	2.01 ± 0.16
Citrus sludge 5.0%	Male	1421.03 ± 36.58	
	Female	1168.44 ± 12.97	
	Mean	1295.40 ± 21.29	1.96 ± 0.04
Citrus sludge 7.5%	Male	1429.42 ± 69.38	
	Female	1161.22 ± 29.92	
	Mean	1295.32 ± 48.16	1.81 ± 0.19
Citrus sludge 10.0%	Male	1541.38 ± 7.07	
	Female	1162.89 ± 39.40	
	Mean	1352.14 ± 23.05	1.97 ± 0.10
Yellow corn '2.5'[d]	Male	1390.74 ± 98.16	
	Female	1124.07 ± 68.52	
	Mean	1257.41 ± 83.12	2.13 ± 0.09
Yellow corn '5.0'	Male	1530.43 ± 25.41	
	Female	1162.32 ± 29.50	
	Mean	1446.38 ± 100.31	1.94 ± 0.02
Yellow corn '7.5'	Male	1577.77 ± 17.39	
	Female	1147.23 ± 2.76	
	Mean	1362.50 ± 9.54	1.93 ± 0.01
Yellow corn '10.0'	Male	1537.73 ± 18.18	
	Female	1171.29 ± 6.69	
	Mean	1354.50 ± 10.95	2.04 ± 0.08

[a] Weight gained from the 4-8 week period.
[b] Feed efficiency for the 4-8 week period.
[c] Standard error of the mean.
[d] The single quote marks (') indicate that the diet contains a xanthophyll level equivalent to the corresponding citrus sludge diet.

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TABLE 25. DWL, EP, AND LUM VALUES FOR SHANK AND SKIN SAMPLES FROM BROILERS FED CITRUS SLUDGE OR YELLOW CORN DIETS AT EQUIVALENT XANTHOPHYLL LEVELS IN EXPERIMENT 2

Treatment	Shanks[a]			Skin[a]		
	DWL (nm)	EP (%)	LUM (%)	DWL (nm)	EP (%)	LUM (%)
White corn	579.2^{bc}	20.11^{a}	63.95^{a}	582.6^{bc}	17.06^{a}	60.94^{ab}
Citrus sludge						
2.5%	579.6^{c}	20.87^{a}	62.91^{a}	584.0^{c}	17.54^{ab}	58.30^{a}
5.0%	579.2^{bc}	22.50^{b}	63.91^{a}	582.5^{abc}	18.03^{ab}	60.23^{ab}
7.0%	579.0^{bc}	22.66^{b}	63.69^{a}	582.1^{ab}	17.88^{ab}	60.55^{ab}
10.0%	579.4^{c}	23.58^{bc}	63.40^{a}	582.5^{abc}	18.39^{b}	60.00^{ab}
Yellow corn						
'2.5'	578.6^{abc}	24.00^{c}	63.64^{a}	582.6^{bc}	17.41^{ab}	61.10^{b}
'5.0'	578.8^{bc}	27.58^{d}	62.11^{a}	582.5^{abc}	19.24^{c}	59.47^{ab}
'7.5'	578.3^{ab}	31.79^{e}	61.73^{a}	582.6^{ab}	20.05^{c}	58.75^{ab}
'10.0'	577.9^{a}	34.52^{f}	62.02^{a}	581.5^{a}	20.50^{d}	59.31^{ab}

[a]Means within each column with different superscripts are significantly different (<0.05).
[b]The single quote marks (') indicate that the diet contained a xanthophyll level equivalent to the corresponding citrus sludge diet.

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